SEROLOGIC TESTS FOR SYPHILIS FOLLOWING SMALLPOX VACCINATION AND INCLUDING REITER PROTEIN COMPLEMENT FIXATION TECHNIC*

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During a recent study of the relative effectiveness of the Reiter Protein Complement Fixation test (RPCF) and other Serologic Tests for Syphilis (STS) it was desired to include as many as possible tests on sera with false positive reactions (1). Recalling previous experience (2) with reactions following vaccination against smallpox, we therefore tested sera from 212 persons, 97 having primary and 115 having accelerated vaccinia. These were university students undergoing physical examinations on admission to school. The median age of the group was 18 years. It is thought that none previously or currently had syphilis. Nevertheless 57 (26.9%) showed some reaction to one or more STS.

Specimens were tested by seven serologic tests for syphilis. For three of the STS (Hinton, Standard Kahn and Kolmer-Wassermann) "old-style lipoidal" antigens were used and for three (Kline, quantitative VDRL, and Kolmer-Wassermann) cardiolipin-lecithin-cholesterol (C-L-C) antigens were used. All six tests were performed according to directions in the USPHS 1955 manual (3). The Reiter Protein antigen (Ryprogen) was used in a one-fifth volume Kolmer test (RPCF) as modified by Bossak *et al.* (4). The six tests using non-treponemal antigens were performed promptly after receipt of the specimens; aliquots of serum were stored frozen (−20°C) and the RPCF tests were performed later (5).

The time interval from vaccination to the first test varied. The results of the 1941 study suggested that optimum reactivity might occur at 15 to 42 days, the highest proportion occurring in the first few days of this period. Sera taken at 19 to 22 days following vaccination were more reactive in the current study than were sera collected later. In both time periods higher reactivity occurred in the group with primary vaccinia and the reactivity persisted longer. Since those showing no reaction to the first test were not retested, we probably have not demonstrated the greatest possible number of reactors.

Subsequent tests on persons with reactive sera were performed at varying intervals. Of those with primary vaccinia nearly half showed no reaction on the next subsequent testing. In the case of those with accelerated vaccinia every subsequent test showed no reaction or less reactivity than the first. In this study less consideration was given to serial testing than in 1941 because the primary purpose of the studies differed. Also, no particular effort was made to follow each reactor to complete seronegativity. However, it was evident that the false reactions persisted longer in those with primary vaccinia as compared with accclerated vaccinia.

The total number of reactive specimens of serum is shown in the tables. Table I shows all the serologic reactions. In Table II the varying degrees of doubtful and positive reactions are totalled and are contrasted with the complete absence of reactivity. Reactivity was observed in sera from 44 of 97 persons with primary vaccinia (45.4%) and 13 of 115 persons having accelerated vaccinia (11.3%). In the 1941 study the number was 43 in 263 cases of primary vaccinia (16%). Some of the difference between the series is undoubtedly due to variation in the time of obtaining specimens but it is nevertheless interesting to compare the results with the several laboratory technics.

The three STS using "old-style lipoidal" antigens (Hinton, Standard Kahn and Kolmer-Wassermann) are in all essentials similar to those 3 tests reported in 1941 but the proportion of reactors in primary vaccinia is greater than in 1941. Two STS (Kline and VDRL) using C-L-C show a lower percentage of reactions than the flocculation tests using "old-style lipoidal" antigens (Hinton and Standard Kahn). Reactions with the Kolmer-Wassermann test were similar in frequency whether the antigen was "old-style" or C-L-C although it is well established that the C-L-C antigen is the more sensitive in detecting syphilitic reagin.

Although the principal purpose of this study

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TABLE I

Results of seven STS on initial blood specimens from 97 persons with primary vaccinia and 115 persons showing an accelerated reaction

Reaction to Vaccinia		Type of Antigen													
		"Old style" lipoidal						Cardiolipin-lecithin-cholesterol						Treponemal	
	Test Method.....	Hinton		Standard Kahn		Kolmer-Wassermann		Kline		Quant. VDRL		Kolmer-Wassermann		RPCF	
	Reactions	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Primary	Negative	57	59.4	72	75.0	89	91.7	76	78.4	90	92.8	89	91.7	97	100.0
	Doubtful (±, 1+)	4	4.2	8	8.3	2	2.1	7	7.2	3*	3.1	3	3.1		
	Positive (2+, 3+)	16	16.6	12	12.5	4	4.1	10	10.3	4†	4.1	2	2.1		
	Positive 4+	19	19.8	4	4.2	2	2.1	4	4.1			3	3.1		
	Total	96‡	100.0	96‡	100.0	97	100.0	97	100.0	97	100.0	97	100.0	97	100.0
Accelerated	Negative	105	92.1	111	96.5	114	99.1	113	98.2	114	99.1	113	98.2	113	99.1
	Doubtful (±, 1+)	2	1.7	3	2.6									1§	0.9
	Positive (2+, 3+)	6	5.3					1	0.9	1†	0.9	1	0.9		
	Positive 4+	1	0.9	1	0.9	1	0.9	1	0.9			1	0.9		
	Total	114‡	100.0	115	100.0	115	100.0	115	100.0	115	100.0	115	100.0	114‖	100.0

* Weakly positive.
† Positive in undiluted serum only.
‡ Test omitted for one person due to insufficient material.
§ RPCF result doubtful ±, repeated RPCF test result negative.
‖ Test result anticomplimentary for one person.

TABLE II

Total serological reactions (positive and doubtful) observed to each of seven STS following primary and accelerated reactions to vaccinia

Antigen Type	Test Method	Course of Vaccinia					
		Primary			Accelerated		
		No. tested	Reactive		No. tested	Reactive	
			No.	%		No.	%
"Old style" Lipoidal	Hinton	96*	39	40.6	114*	9	7.9
	Standard Kahn	96*	24	25.0	115	4	3.5
	Kolmer-Wassermann	97	8	8.2	115	1	0.9
Cardiolipin-cholesterol-lecithin	Kline	97	21	21.6	115	2	1.7
	Quant. VDRL	97	7	7.2	115	1	0.9
	Kolmer-Wassermann	97	8	8.2	115	2	1.7
Treponemal	RPCF	96†	0	0.0	114†	1‡	0.9

* Insufficient material for one test.
† One test anticomplementary.
‡ Doubtful reaction; second test negative later on the same specimen.

was the comparative evaluation of the RPCF and other STS, it is also of interest to note the frequency with which a single specimen of serum showed reactivity in tests with different types of antigens, a serologic feature which could lead to diagnostic errors in practice (Table III). Particularly in primary vaccinia the proportion of persons showing reactivity to four or more

TABLE III

Distribution of persons according to the number of STS which were reactive† and the days elapsed between vaccination and collection of initial blood specimen*

Reaction to Vaccinia	Number of Seven STS* Reactive†	Days Elapsed Between Vaccination and Collection of Initial Blood Specimen			Total	
		19–22	23–36	37–52		
		No. Persons	No. Persons	No. Persons	Persons	
					No.	%
Primary	None	1	30	22	53	54.6
	One	4	11	2	17	17.5
	Two	5	3	0	8	8.3
	Three	1	6	3	10	10.3
	Four to six	2	6	1	9	9.3
	Total	13	56	28	97	100.0
Accelerated	None	23	80		103	89.5
	One	7	1		8	7.0
	Two	2	1		3	2.6
	Three	0	0		0	0.0
	Four to six	1	0		1	0.9
	Total	33	82		115	100.0

* Seven STS (see text) were performed on initial specimens from all except 2 persons.

† Including doubtful and positive reactions but not including anticomplementary RPCF result on one, and three tests (1 Kahn, 2 Hintons) for which there was insufficient material.

tests is distressingly high. Review of data from several cases shows clearly the need for alert and instructed clinicians:

Case I. P. W. B., 18 year old male with primary vaccinia, first specimen at 23 days.

Kl.	VDRL	Kol.	C. Kol.	K.	H.	RPCF	No. Days
4+	+ undil. only	4+	4+	4+	4+	—	23
2+	wkly. pos.	3+	3+	3+	4+	—	37
Dbt. ±	—	—	—	1+	4+	—	52

Without knowledge of the patient's primary vaccinia a diagnostic error could result here from the high degree of reactivity to five different antigens if one did not have available the report of a doubtful degree of reactivity to the VDRL and the RPCF result.

Case II. J. M. L., 18 year old male with accelerated vaccinia, first specimen at 22 days.

Kl.	VDRL	Kol.	C. Kol.	K.	H.	RPCF	No. Days
4+	+ undil. only	4+	4+	4+	4+	—	22
Dbt. ±	—	3+	4+	4+	4+	—	36
—	—	3+	4+	2+	3+	—	51
—	—	Dbt. ±	4+	2+	1+	—	65
—	—	—	4+	1+	—	—	79
—	—	3+	4+	1+	—	—	95
—	—	—	—	1+	—	—	112

In examining a patient with accelerated vaccinia the immunization procedure might be overlooked or ignored more easily than with a primary take but here, too, reactivity can occur at a high level and to many different technics.

Case III. A. D. S., 19 year old male with primary vaccinia, specimen at 29 days.

Kl.	VDRL	Kol.	C. Kol.	K.	H.	RPCF	No. Days
—	—	4+	4+	3+	4+	—	29

This case illustrates the same points but shows strong reactivity to only four antigens in sharp contrast with complete absence of reaction in other tests.

More often the results of testing are less confusing to the clinician, showing a bizarre pattern of reactivity which is generally recognized as likely to represent "false" positivity or to occur following treatment for syphilis:

Case IV. R. E. S., 18 year old male with primary vaccinia, first specimen at 23 days.

Kl.	VDRL	Kol.	C. Kol.	K.	H.	RPCF	No. Days
4+	+ undil. only	3+	1+	4+	4+	—	23
2+	wk. +	—	—	—	4+	—	37
Dbt. ±	—	—	—	—	1+	—	52
—	—	—	—	—	2+	—	66
—	—	—	—	—	—	—	80

Case V. M.T.C., 19 year old female with primary vaccinia, first specimen at 32 days.

In each of the above cases and in the cases of many less reactive sera the clinician would be greatly helped by the knowledge that the RPCF

Kl.	VDRL	Kol.	C. Kol.	K.	H.	RPCF	No. Days
Dbt. ±	—	3+	4+	1+	4+	—	32
—	—	—	—	—	4+	—	49
—	—	—	—	—	—	—	64

technic showed absolutely no reactivity. Among 210 specimens, the RPCF test was non-reactive on 209 and weakly reactive on one (0.5%). The RPCF test was the only reactive test on this individual and when the RPCF test was repeated on the same specimen at a later date the RPCF test was non-reactive.

Case VI. B.T.G., 20 year old male with accelerated vaccinia, specimen at 20 days.

Kl.	VDRL	Kol.	C. Kol.	K.	H.	RPCF	No. Days
—	—	—	—	—	—	±	20

This lack of repeatability suggests two possibilities: that the reaction was due to technical error or that the reaction was due to a (very low) potentiality for vaccinia to produce BFP reactions with the RPCF test. The RPCF test was the only reactive test observed with this specimen. This fact might be interpreted as supporting the possibility of a technical error but it does not exclude the alternate possibility that it was a weak BFP reaction. It would be strange to have a BFP reaction in the presence of accelerated vaccinia in this instance and not in any case with primary vaccinia. In either case the weakly positive reaction on the first test surely underlines the need for continual attention to basic serodiagnostic rules:

1. In routine examinations the serologic specimen should be obtained prior to the institution of immunologic procedures.

2. The serologic battery should include several tests using different types of antigen and having varying degrees of sensitivity and specificity.

3. Without the strongest of clinical evidence no diagnosis should be based on tests done on only one specimen of serum.

4. Unless in a treated case, where a diagnosis was clearly established previously, doubtful or positive-reacting sera on standard STS should be re-tested by one or more technics utilizing a treponemal antigen.

It is encouraging that the results of this study suggest that the solution of serodiagnostic problems may be aided by the use of the RPCF test, a relatively inexpensive but dependable test using a treponemal antigen.

CONCLUSIONS

Serum specimens collected after recent vaccination were submitted to a battery of STS with comparative study of the false reactors with the RPCF technic and the older STS.

In a series of 212 persons with vaccinia there were 57 false reactors to other STS and only one serum specimen (of 210 tested) reacted to the RPCF test; this was only a doubtful reaction and when the same specimen was re-tested there was complete absence of reaction. This study thus supports the claims of many workers that the RPCF test has a high degree of specificity.

EDITOR'S NOTE: This paper was discussed jointly with that of William J. Brown, Eleanor V. Price and W. G. Simpson, "The Reiter Protein Antigen Test Compared With The TPI and Other Treponemal and Nontreponemal Antigen Technics in the Diagnosis of Syphilis," which follows immediately.

REFERENCES

1. a. BAUER, HENRY AND PINKE, K. YVONNE: Laboratory evaluation of the Reiter Protein Complement Fixation Test in the diagnosis of syphilis. Minnesota Med., **43:** 1–5 1960.
   b. LYNCH, FRANCIS W. AND BINDER, R. H., JR.: Clinical evaluation of the Reiter Protein Complement Fixation Test in the diagnosis of syphilis. Minnesota Med., **43:** 6–10, 1960.
2. LYNCH, FRANCIS W., BOYNTON, RUTH E. AND KIMBALL, ANNE C.: False positive serologic reactions for syphilis—due to smallpox vaccinations (Vaccinia). J.A.M.A., **117:** 591, 1941.
3. U. S. Public Health Service: Serologic Tests for Syphilis, 1955 manual. PHS No. 411 Washington D. C., U. S. Government Printing Office, 1955.
4. BOSSAK, H. N., FALCONE, V. H., DUNCAN, W. P. AND HARRIS, A.: Kolmer test with Reiter Protein Antigen; Venereal Disease Research Laboratory Modification. Public Health Laboratory **16:** 39, March 1958.
5. One RPCF test result was anticomplementary although the two Kolmer-Wassermann complement fixation test results on the same specimen were not anticomplementary. This inconsistence was probably due to the fact that the Kolmer-Wassermann was performed on fresh serum and the RPCF on serum which had been stored frozen. Freezing and thawing occasionally enhances the anticomplementary quality of serum specimens.